Diverging Evolution of Anti-GAD and Anti-IA-2 Antibodies in Long-standing Diabetes Mellitus as a Function of Age at Onset: No Association with Complications

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Glutamic acid decarboxylase autoantibodies (GAD-A) and tyrosine phosphatase IA-2 autoantibodies (IA2-A) were measured in sera of 50 recently diagnosed (<6 wk, 33 % younger than 15 yr), 19 short-term (1 to 9 yr, 35 % with onset age below 15 yr) and 89 long-standing diabetic patients (>10 yr, 57 % with onset age below 15 yr). Complications were assessed by clinical examination, retinal angiographs and microalbuminuria measurement. Both prevalences and levels of GAD-A and IA2-A decreased with increasing duration of diabetes. However even in those with long duration diabetes, 15 to 63 % of the sera were still positive for one or two antibodies. In the group with onset after the age of 15 yr, significantly higher prevalences and levels of GAD-A (but not IA2-A) was observed in comparison with the group with earlier onset. No association was found with any microvascular complications in any group. We conclude that GAD-A and IA2-A persist in some diabetic patients, despite a long duration. Persistence of GAD-A was greatest in those with postpubertal disease onset. We speculate that persistence of some beta-cells or specific environmental factors can sustain one autoimmune reaction especially in some postpubertal-onset diabetic patients. © 1998 John Wiley & Sons, Ltd.

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Introduction

The natural course of Type 1 diabetes mellitus can differ according to the age of onset and particularly whether occurring before or after puberty. MHC genotypes[1,2] and autoantibodies have been reported to be different in prepubertal-onset disease.[1,3,4] In addition, puberty can be a risk for the future development of microvascular complications. Indeed a higher prevalence of retinopathy and nephropathy has been reported in populations of pubertal-onset Type 1 DM patients compared to prepubertal-onset patients at a given duration.[5,6] According to Kostraba, puberty is an important step in the development of microvascular complications and the risk of premature mortality in Type 1 diabetic patients.[7]

In the present study, two populations of Type 1 diabetic patients were defined by age of onset, before or after 15 years, and duration of disease. The groups were compared in terms of prevalence of autoantibodies directed to chemically defined antigens, i.e. glutamic acid decarboxylase (GAD-A) and pancreatic tyrosine phosphatase IA-2 (IA2-A). A relationship between the presence of microvascular complications with the anti-islet humoral reaction was sought in the patients with the longest disease duration.

Patients and Methods

Patients

We recruited 50 recently diagnosed Type 1 DM patients, defined by having a duration of symptoms of less than 6 weeks. Seventeen patients (30 %) had an age at onset of disease of less than 15 years. Nineteen Type 1 DM

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patients, duration of disease between 1 and 9 years and all without complications (short-term patients) and 89 long-standing diabetic patients (disease duration more than 10 years) were also recruited. In the latter two groups, the percentage of below 15 yr-onset patients were 36.8 % (7/19) and 57.3 % (51/89) respectively. Among the long-standing diabetic patients, 22 had no complications (24.72 %) and 67 had one or more of retinopathy, nephropathy or neuropathy.

The different diabetic complications were defined as published.[8] On the basis of fundus ophthalmoscopy and fluoroscein angiology, the severity of diabetic retinopathy was classified into three categories: no, moderate, severe. Diabetic nephropathy was absent if urinary albumin excretion (nephelometric assay) was lower than 30 mg per day on two occasions, incipient if it was between 30 and 300 mg per day and overt when macroalbuminuria was present. Neuropathy was classified as severe if two or three of the Diabetes Control and Complication trial (DCCT) criteria[9,10] were affected, mild if one of the three criteria was met, and absent if only one of these criteria was found. As no differences were observed between various degrees of severity in each complication, the results were presented after pooling background and proliferative retinopathies, micro- and macroalbuminuria, mild and severe neuropathies.

Assay for GAD and IA-2 Autoantibodies

GAD_{65} and IA-2 antibodies were detected by radiobinding assays with [^{35}S]-methionine labelled recombinant human GAD_{65} and IA-2 antigens obtained by *in vitro* translation, as previously described.[4] GAD_{65} cDNA and IA-2 cDNA were kindly provided by Dr E. Bonifacio, Instituto Scientifico San Raffaele, University of Milan, Italy. Radioactivity was expressed in cpm. The upper limit of normal range (3 SD above normal mean) for GAD_{65} and IA2 antibodies was 200 cpm and 190 cpm, respectively. The results of GAD antibodies assay in the third GAD proficiency test were 100 % validity, 100 % consistency, 100 % sensitivity, 100 % specificity.

Statistical Analysis

Results were expressed by mean ± SEM. Differences in GAD-A or IA2-A prevalence between study groups were tested with the χ^2 test. Differences in antibody titres were analysed by ANOVA; $p < 0.05$ was considered to be statistically significant.

Results

Prevalence of GAD-A and IA2-A in Recently Diagnosed Type 1 DM Patients

The prevalences of GAD-A and IA2-A at diagnosis were 94 % and 52 %, respectively. When two groups of recently diagnosed patients were divided according to age before and after 15 years (Table 1), a trend towards a higher prevalence of GAD-A and a lower prevalence of IA2-A was observed in the older group but these differences did not reach statistical significance (97 vs 88 % and 45 vs 65 %, respectively). The frequency of double positivity was similar in the two groups (52.9 vs 42.4 %). The level of GAD-A was significantly higher in the older onset group (4331 ± 556 vs 2819 ± 612 cpm, $p < 0.05$). No difference was observed between the two groups for IA2-A (3035 ± 790 vs 2442 ± 1097, respectively).

Table 1. Prevalence of GAD_{65}-A and IA2-A according to age at Type 1 DM clinical onset in different Type 1 DM groups: recent onset, short-term, long-term with and without diabetic complications

	Recently diagnosed Type 1 DM patients		Short-term Type 1 DM patients		Long-standing Type 1 DM patients	
Age at Type 1 DM onset (yr)	≤15	>15	≤15	>15	≤15	>15
Mean ± SEM	9.59 ± 1.04	24.12 ± 1.02	8.46 ± 0.93	22.83 ± 1.15	8.62 ± 0.59	23.76 ± 0.81
(range)	(2–15)	(16–38)	(6–12.5)	(17–30)	(1–15)	(16–35)
Diabetes duration	<6 wk after diagnosis		3.79 ± 1.16	3.38 ±0.94	23.10 ± 1.32	20.47 ± 1.41
Mean ± SEM (yr)			(1–9)	(1–9)	(10–48)	(10–43)
GAD_{65} prevalence	15/17	32/33	4/7	10/12	20/51[c]	24/38[c d]
	(88)	(97)	(57)	(83)	(39.2)	(63.1)
Levels (cpm)	2819	4331	1438	3415	931	1931
	+/–612	+/–557[d]	+/–694	+/–821[e]	+/–254[a]	+/–417[a e]
IA2 prevalence	11/17	15/33	4/7	4/12	14/51[a]	6/38[a]
	(65)	(45)	(57)	(33)	(27.5)	(15.8)
Levels (cpm)	2442	3035	1817	1428	717	848
	+/–1097	+/–790	+/–1390	+/–828	+/–207	+/–396
Double positivity	9/17	14/33	2/7	4/12	7/51	5/38
	(52.9)	(42.4)	(28.6)	(33.3)	(13.7)	(13.2)

Comparison between recent-onset Type 1 DM and short-term or long-term Type 1 DM groups belonging to the same age group [a]$p < 0.05$, [b]$p < 0.005$, [c]$p < 0.0005$).
Comparison between (≤15 yr and 15 yr) onset Type 1 DM age groups [d]$p < 0.05$, [e]$p < 0.01$.

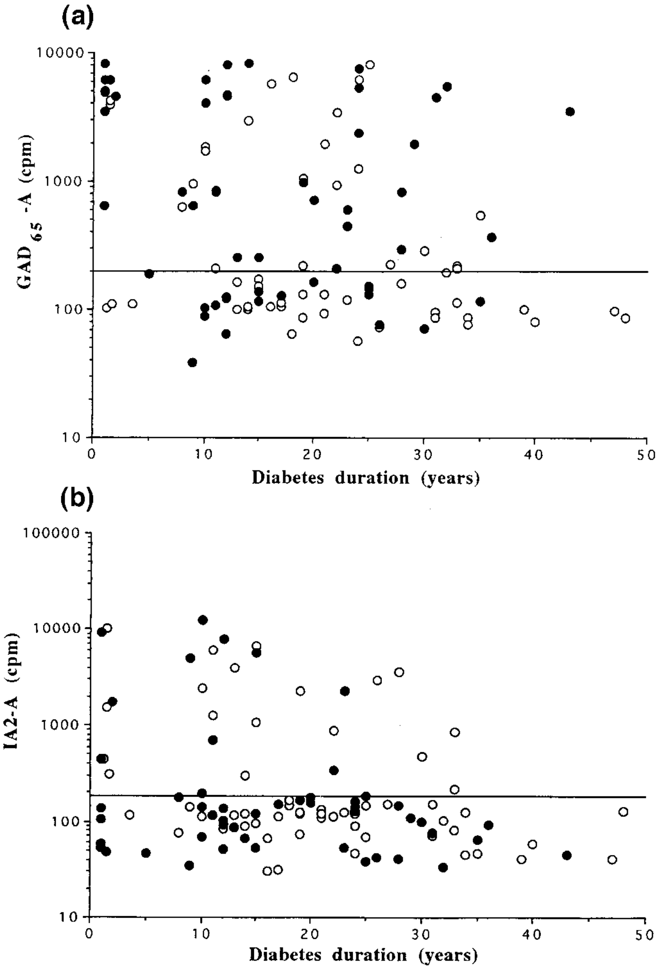

Figure 1. Distribution of (a) GAD$_{65}$-A and (b) IA2-A according to diabetes duration, in 108 Type 1 DM patients; ○ age at Type 1 DM clinical onset ≤ 15 years (mean ± SD: 8.61 ± 4,00; range 1–15), $n = 58$; ● age at Type 1 DM clinical onset > 15 years (mean ± SD: 23.38 ± 4.86; range 16–35), $n = 50$. Horizontal line represents the upper limit of the normal range

Levels and Prevalences of GAD-A and IA2-A in Established diabetes as a Function of Age of Onset

Both antibodies were less prevalent in the patients with longer duration diabetes. Positive GAD-A was more frequent in these groups than IA2-A. In the postpubertal-onset group with a diabetes duration >10 years, GAD-A was more frequently positive than in the prepubertal-onset one (63.2 vs 39.2 % $p < 0.05$). In these groups double positivity was observed at the same rate (13 %).

The binding activities for both antibodies are presented in Figure 1. In the two groups some sera displayed a high level of GAD-A or IA2-A despite long duration of diabetes. Significantly higher levels of GAD-A were observed in the >15 yr-onset group whatever the diabetes duration ($p < 0.05$ at diagnosis, $p < 0.01$ at 1–9 years and $p < 0.01$ after 10 years). In contrast no significant difference was noted for IA2-A between the two groups.

GAD-A and IA2-A According to the Presence of Microvascular Complications

As shown in Table 2, the prevalence of GAD-A and IA2-A were not influenced by the presence or absence of microvascular complications. Even in subgroups perfectly matched for the duration of clinical diabetes (10 to 25 years) no difference was observed in the frequency of GAD-A and IA2-A. In the < 15 yr-onset group, 10 patients had no detectable complications, and were 40 % GAD-A and 30 % IA2-A positive, respectively; 16 showed one or more complications, and were 56 % GAD-A and 19 % IA2-A positive, respectively. In the > 15 yr-onset, 10 patients were exempt of complications and were 50 % GAD-A and 30 % IA2-A positive, respectively, 25 showed one or more microvascular complications, and were 44 % GAD-A and 24 % IA2-A positive, respectively. The lack of association was also noted for each complication, retinopathy, nephropathy or neuropathy. A subgroup of 25 patients exhibiting retinopathy but neither neuropathy nor nephropathy did not differ from the uncomplicated group in terms of antibodies. There was also no relationship between complications and levels of antibodies present in serum.

Discussion

This study confirms that antibodies directed to chemically defined islet antigens, GAD and IA-2 can persist in Type 1 diabetic patients even after a long duration of the disease.[11–14] The prevalence of GAD-A and IA2-A after 10 years in this study is higher than that previously observed for classical islet cell antibodies.[12,15,16] This discrepancy is probably due to the greater sensitivity of this assay. Indeed it is noteworthy that in our long-standing diabetic population the prevalence of double positivity (13 %) is close to that reported for ICA (15 to 20 %).[12,15,16] We found that the prevalence of GAD-A was affected by the age of onset of the diabetes, irrespective of disease duration. Patients with a postpubertal onset of diabetes exhibited a higher frequency and a higher level of GAD-A. In contrast the prevalence and the level of IA2-A was not significantly different between groups with different age of onset. Some authors have shown that IA2-A are more frequently found in diabetes with an onset in children.[4,17–19] Results are more conflicting for GAD-A. Measuring samples taken at disease onset, some authors observed a higher frequency of GAD-A in adults,[4,17] others in children.[18,19] Very few studies have examined the effect of age of onset on the persisting antibodies after long duration. In agreement with our data, Roll observed a higher prevalence of GAD-A in long-standing diabetes.[12] Two explanations of the persistence of such antibodies can be proposed. First, it is possible that very few residual β-cells are able to sustain the autoimmune reaction. According to Yokota, the β-cell response to glucagon stimulation is better at 3 and 5 years in patients remaining GAD-A positive,

Table 2. Prevalence of GAD_{65}-A and IA2-A according to age at Type 1 DM clinical onset in long-term Type 1 DM patients with retinopathy and/or nephropathy and/or neuropathy

	Diabetic patients without complications		Diabetic patients with retinopathy associated or not with nephropathy and/or neuropathy		Diabetic patients with nephropathy associated or not with retinopathy and/or neuropathy		Diabetic patients with neuropathy associated or not with retinopathy and/or nephropathy	
Age at Type 1 DM onset (yr)	≤15	>15	≤15	>15	≤15	>15	≤15	>15
Mean ± SD	10.09 ± 4.32 (2–15)	24.36 ± 4.56 (16–35)	8.62 ± 4.13 (1–14)	23.48 ± 5.30 (16–35)	9.07 ± 4.01 (1–14)	23.42 ± 6.08 (16–35)	8.06 ± 3.65 (2–14)	24.07 ± 5.90 (16–35)
Diabetes duration mean ± SD (yr) (range)	16.9 ± 6.11 (10–32)	15.72 ± 5.97 (10–28)	25.08 ± 9.42 (10–48)	22.60 ± 8.56 (10–43)	23.46 ± 6.46 (14–34)	22.42 ± 10.63 (10–43)	27.35 ± 7.78 (14–48)	24.71 ± 7.38 (10–36)
GAD-A	5/11 (45)	6/11 (55)	14/38 (37)	17/25 (68)	5/13 (38)	9/12 (75)	6/7 (35)	10/14 (71)
IA2-A	3/11 (27)	3/11 (27)	11/38 (29)	2/25 (8)	3/13 (23)	2/12 (17)	4/17 (24)	1/14 (7)

Results of GAD_{65} and IA2 autoantibodies are *n* or (%).
χ^2 tests were performed between new onset Type 1 DM age groups (≤15 yr and 15 yr).

suggesting a link between residual β-cell presence and GAD-A.[20] Second, some environmental agents may maintain pre-existing immune processes. Our observation, specific for GAD-A, favours the second hypothesis and suggests that the environmental factors predisposing to diabetes might be different before and after puberty. Among the environmental agents which have been implicated in the pathogenesis of Type 1 diabetes, coxsackie B4 is a strong candidate. The sequence homology between P2-C protein of coxsackie B4 virus and GAD[21] as well as epidemiological data[22,23] suggest that coxsackie virus could induce and/or sustain anti-GAD humoral response by mimicry. The data on coxsackie virus IgM serology in recent-onset diabetes according to age of onset are conflicting. Banatvala[22] observed positive serology more frequently in patients with onset before the age of 15, but the number of older patients was small. In contrast, Karjalainen[23] found inverse associations between ICA or IAA and age at onset and between ICA and viral antibodies in a large series of diabetic patients. These data suggest that autoimmunity may play a more crucial role in younger patients contracting diabetes, while environmental factors may be more important in older ones.

The present study was unable to find any relationship between persisting GAD-A or IA2-A and microvascular complications, even taking account of the age of onset before or after puberty. We may not have had the power to decide this but our negative results confirm previous reports[11–15] (but one[24]) of a lack of relationship between GAD-A and either neuropathy or other microvascular complications. They are also in agreement with the data of Muhr[15] and Zerbini[14] who did not find any higher frequency of IA2-A in patients with autonomic cardiac neuropathy or nephropathy, respectively.

In conclusion, GAD-A and IA2-A are surprisingly frequent in long-standing diabetic patients. Persisting GAD-A are even more prevalent when diabetes has been diagnosed after the age of 15 years. Persistence of some residual β-cells and/or specific environmental agents may be involved in the persistence of the autoimmune processes in some patients, and this is especially true for those with postpubertal onset of the disease.

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